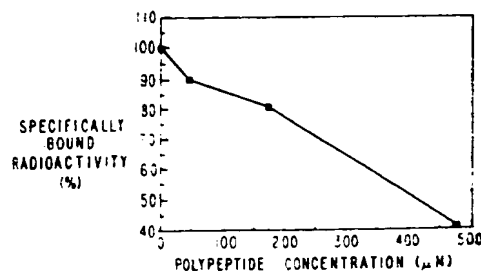
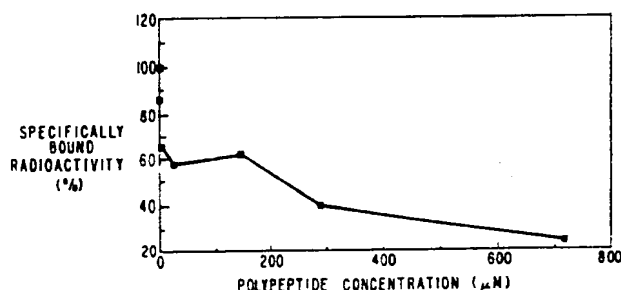




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(54) Title: ANTAGONISTS OF HUMAN GAMMA INTERFERON



(57) Abstract

Novel synthetic polypeptides having amino acid sequences corresponding to the sequence of one or more specific regions of human gamma interferon are provided by this invention. These polypeptides specifically inhibit the binding of human gamma interferon to cellular receptors and the biological activity of such interferon. Antibody antagonists of the binding of human gamma interferon to cellular receptors based upon the use of the polypeptides as antigens are also provided. Some of these antagonists bind to specific regions of gamma interferon which are believed to be involved in interactions between the interferon and its receptors. Other antibody antagonists are anti-idiotypic antibodies which appear to compete directly with gamma interferon for binding to the cellular receptors. Also provided are methods for the use of the polypeptides and antibodies as inhibitors of the binding of gamma interferon to its cellular receptors.

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ANTAGONISTS OF HUMAN GAMMA INTERFERON

5

BACKGROUND OF THE INVENTION

Gamma interferon is a protein produced by activated helper T cells which manifests antiviral, antiproliferative and immunomodulatory activities. The
10 antiviral and antiproliferative activities of gamma interferon are largely suppressive, while the immunomodulatory activity is primarily expressed through stimulation of a variety of immune functions, although the inhibition of immune function is also known to occur.

15 Although the mechanism by which gamma interferon exerts its effects on cells is not understood, it is known that it binds to specific cellular receptors [Langer *et al.*, Immunology Today 9:393 (1988)]. Aguet *et al.* [Cell 55:273 (1988)] have cloned and sequenced a gene for a gamma
20 interferon receptor. The molecular weight of the encoded protein deduced from the sequence is consistent with the molecular weight of a gamma interferon receptor recently isolated from human placenta [Calderon *et al.*, Proc. Natl. Acad. Sci. USA 85:4837 (1988)].

25 Gamma interferon is believed to be involved in autoimmune disease. Elevated levels of gamma interferon are also believed to stimulate macrophages that erroneously digest myelin in the brain and spinal cord of multiple sclerosis (MS) sufferers.

Because gamma interferon acts at specific cellular receptors and is implicated in autoimmune disease and MS, agents which could inhibit the binding of such interferon to its cellular receptors would be useful therapeutically.

SUMMARY OF THE INVENTION

This invention provides novel polypeptides containing up to 50 amino acid residues and comprising one or more amino acid subsequences selected from the group consisting of the subsequences defined by amino acid residues 15-21 and 132-137 of the sequence defined by SEQ ID NO:1. Preferably the polypeptides contain up to 40, and more preferably up to 35, amino acid residues.

In some polypeptides comprising both of the subsequences, the subsequences are separated by a peptide linker containing from 1 to about 20 amino acid residues. In other polypeptides comprising both subsequences, the subsequences are in two separate polypeptides joined by disulfide bridges.

This invention further provides antibodies against polypeptides containing up to 50, preferably up to 40 and more preferably up to 35, amino acid residues and comprising one or more amino acid subsequences selected from the group consisting of the subsequences defined by amino acid residues 15-21 and 132-137 of the sequence defined by SEQ ID NO:1, which antibodies are characterized by an ability to specifically bind to the polypeptides and to human gamma interferon and to inhibit the binding of such interferon to cellular receptors.

30

The present invention still further provides anti-idiotypic antibodies against the above-mentioned antibodies. These anti-idiotypic antibodies also inhibit the binding of human gamma interferon to cellular receptors.

5 All of the antibodies of this invention can be either polyclonal antibodies from an antiserum, or monoclonal antibodies. Binding fragments made from such antibodies and humanized or otherwise engineered antibodies are also a part of this invention.

10 Also provided are methods for using the foregoing polypeptides and antibodies to inhibit the binding of such interferon to cellular receptors.

One method comprises contacting cells bearing receptors for human gamma interferon with an effective
15 amount of a polypeptide containing up to 50, preferably up to 40 and more preferably up to 35, amino acid residues and comprising one or more amino acid subsequences selected from the group consisting of the subsequences defined by amino acid residues 15-21 and 132-137 of the sequence
20 defined by SEQ ID NO:1.

Another method comprises contacting human gamma interferon with an effective amount of an antibody which specifically binds to human gamma interferon and to a polypeptide containing up to 50, preferably up to 40 and more
25 preferably up to 35, amino acid residues and comprising one or more amino acid subsequences selected from the group consisting of the subsequences defined by amino acid residues 15-21 and 132-137 of the sequence defined by SEQ ID NO:1.

Still another method comprises contacting cells
30 bearing receptors for human gamma interferon with an effective amount of a first, anti-idiotypic, antibody against a

second antibody, which second antibody specifically binds to human gamma interferon and to a polypeptide containing up to 50, preferably up to 40 and more preferably up to 35, amino acid residues and comprising one or more amino acid
5 subsequences selected from the group consisting of the subsequences defined by amino acid residues 15-21 and 132-137 of the sequence defined by SEQ ID NO:1.

In addition to their use as therapeutic agents, the polypeptides, antibodies and methods of the present invention
10 are useful for the *in vitro* study of the mechanism of gamma interferon binding to various cell types and for screening for other gamma interferon antagonists or agonists.

BRIEF DESCRIPTION OF THE FIGURES

This invention can be more readily understood by
15 reference to the accompanying figures, in which:

Fig. 1 is a graphical representation of the inhibition of the binding of ^{125}I -labeled human gamma interferon D to Daudi cells by polypeptides having amino acid sequences defined by SEQ ID NO:2 (panel A) and by SEQ ID NO:3
20 (panel B). Percent specifically bound radioactivity is shown in both panels as a function of polypeptide concentration.

Fig. 2 is a graphical representation of the inhibition of the binding of ^{125}I -labeled human gamma interferon D to Daudi cells by antiserum against the
25 polypeptide mentioned in the legend to panel A of Fig. 2, showing percent specifically bound radioactivity as a function of the amount of antiserum.

Fig. 3 is a graphical representation of the inhibition by a polypeptide having an amino acid sequence
30 defined by SEQ ID NO:10 of the induction of class II major

histocompatibility antigens on COLO-205 cells by recombinant human gamma interferon D. Percent inhibition is shown as a function of polypeptide concentration in the presence of a constant 150 pM concentration of interferon.

5

DESCRIPTION OF THE INVENTION

All references cited herein are hereby incorporated in their entirety by reference. All amino acid sequences disclosed follow the normal convention, with the amino terminus on the left and the carboxyl terminus on the right. Standard three-letter abbreviations are used for the amino acid residues in the sequences (37 C.F.R. § 1.822). As used herein, "Aib" represents either 2- or 3-aminoisobutyric acid.

In the course of a search for specific inhibitors of human gamma interferon, it has surprisingly been found that certain polypeptides having amino acid sequences corresponding to the sequences of residues in specific regions of intact mature human gamma interferon are potent inhibitors of the binding of gamma interferon to its cellular receptors. The antagonistic actions of these polypeptides have been demonstrated in a radioligand-receptor assay system employing the polypeptides, ¹²⁵I-labeled human gamma interferon and cells bearing specific receptors for such interferon. This invention provides such polypeptides.

The present invention also provides antibodies which inhibit the binding of human gamma interferon to cellular receptors by (a) combining with a region(s) in such interferon that apparently is involved in interactions with the receptors or by (b) mimicking gamma interferon itself, thereby competing with it for binding to the receptors. As a result, they also inhibit the biological activity of the interferon.

As used herein, human "gamma interferon" means a protein which (a) has an amino acid sequence substantially identical to the sequence of mature human gamma interferon A as defined in the Sequence Listing by SEQ ID NO:1 and (b) has biological activity that is common to native gamma interferon.

For convenience, the amino acid sequences of polypeptides mentioned below may be described in terms of the corresponding sequences of residues in the amino acid sequence of mature human gamma interferon defined by SEQ ID NO:1, with 1 being the amino-terminal cysteine residue and 146 being the carboxyl-terminal glutamine residue, etc.

Substantial identity of amino acid sequences means that the sequence of another gamma interferon compared to the sequence defined by SEQ ID NO:1 is identical or differs by one or more amino acid alterations (deletions, additions, substitutions) that do not substantially impair biological activity. For example, gamma interferon D, which lacks the first three amino-terminal residues (Cys-Tyr-Cys) of the interferon defined by SEQ ID NO:1, is substantially identical in the context of this invention. So too are the natural human gamma interferons which lack such amino-terminal residues and in addition display microheterogeneity at the carboxyl terminus [Seelig *et al.*, Biochemistry 27:1981 (1988)].

As explained above, the polypeptides of the invention comprise one or more amino acid subsequences selected from the group consisting of the subsequences defined by amino acid residues 15-21 and 132-137 of the sequence defined by SEQ ID NO:1. Preferred polypeptides comprise both. These two subsequences comprise important "core regions" of human gamma interferon which are believed

to be somehow involved in the receptor binding and/or biological activity of such interferon. The polypeptides may contain, in addition to these core regions, additional flanking sequences comprising amino acid residues having sequences
5 corresponding to the sequences of residues flanking the core regions in human gamma interferon.

For example, a preferred polypeptide having an amino acid sequence defined by SEQ ID NO:10 comprises two subsequences, one of which is defined by the sequence of
10 amino acid residues 15-29 of SEQ ID NO:1. The other subsequence in this polypeptide is defined by the sequence of amino acid residues 130-138 of SEQ ID NO:1.

When the polypeptides of the invention contain both of the core regions (with or without additional flanking
15 sequences), the regions are brought into proximity with each other in one of two ways. In a preferred embodiment, they are covalently joined in peptide linkage by an intervening linker peptide. This linker peptide can contain from 1 to about 20
20 amino acid residues, preferably from about 3 to about 8 residues. The amino acid residues selected for the linker peptide can be randomly selected from any of the 22 commonly known amino acids, although amino acid residues selected from the group consisting of Gly, Ala, Aib, Leu and Ile are preferred for use in random-sequence linkers.

25 Instead of randomly-selected amino acid residues, the linker peptide may contain from 1 to about 20, preferably from about 3 to about 8, residues having a sequence corresponding to the sequence of a subsequence of residues present in human gamma interferon between the two core
30 regions. For example, in the preferred embodiment defined by SEQ ID NO:10, the two regions are joined by a peptide linker

having a sequence corresponding to the sequence of residues 111-118 of SEQ ID NO:1.

5 The linker peptide is provided to confer flexibility on the molecule, so that the regions related to the interferon sequences may have a spatial relationship which approximates the spatial relationship of the corresponding sequences in intact human gamma interferon. It will of course be appreciated by those skilled in the art that this objective can also be accomplished with linkers comprising 10 units other than amino acid residues. For example, many of the well-known linkers commonly employed in affinity chromatography can be used instead, as long as the flexibility and length of the linkers are similar to those of the polypeptide linkers. Such functionally equivalent, alternative 15 linkers can be attached to the polypeptide segments of the molecules by known methods.

The order of the core regions (with or without additional flanking sequences) in the foregoing polypeptides is not essential. The region located in the amino-terminal 20 region of human gamma interferon can be at the amino terminus of the polypeptide, or vice versa, although the former arrangement is preferred.

Some preferred polypeptides containing one or both of the core regions have amino acid sequences defined in 25 the Sequence Listing by SEQ ID NOs. 2, 3 and 10.

In an alternative embodiment, the two core regions (with or without additional flanking sequences) can be brought into proximity by using two polypeptides, each of which contains one of the core regions. This is accomplished 30 by incorporating cysteine residues into each of the polypeptides in regions outside of the core regions, and joining the polypeptides by disulfide bonds.

As used herein, the term "polypeptide" is defined to mean both embodiments in which the core regions are brought into proximity via a linker peptide, and those in which two polypeptide chains are joined by disulfide bridges.

5 Although the polypeptides of the invention contain only a relatively small number of amino acid residues compared to the total number of residues in mature human gamma interferon, they are nevertheless specific competitive
10 inhibitors of the binding of the intact interferon to cellular receptors. As shown below, as much as 80% of the specific binding of ¹²⁵I-labeled human gamma interferon D to Daudi cells can be abolished by such polypeptides.

 It must be stressed that the polypeptides of the invention will bind to any cells which have gamma interferon
15 receptors, such as B cells, T cells, eosinophils, smooth muscle cells, promyelocytes, macrophages, erythroid cells, monocytes and granulocytes. Daudi cells, a well-characterized B lymphoblast cell line derived from a Burkitt lymphoma patient which are available from the American Type Culture
20 Collection under Accession No. CCL 213, are used below merely as a convenient way to demonstrate the inhibition of the binding of ¹²⁵I-labeled gamma interferon to cellular receptors. Other cell lines can also be used for this purpose, such as the U-937 human histiocytic lymphoma line (ATCC CRL 1593).

25 The polypeptides are synthesized by a suitable method such as by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The polypeptides are preferably prepared by solid phase peptide synthesis as described by Merrifield,
30 J. Am. Chem. Soc. 85:2149 (1963). The synthesis is carried out with amino acids that are protected at the alpha-amino terminus. Trifunctional amino acids with labile side-chains

are also protected with suitable groups to prevent undesired chemical reactions from occurring during the assembly of the polypeptides. The alpha-amino protecting group is selectively removed to allow subsequent reaction to take place at the amino-terminus. The conditions for the removal of the alpha-amino protecting group do not remove the side-chain protecting groups.

The alpha-amino protecting groups are those known to be useful in the art of stepwise polypeptide synthesis. Included are acyl type protecting groups (e.g., formyl, trifluoroacetyl, acetyl), aromatic urethane type protecting groups [e.g., benzyloxycarbonyl (Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxycarbonyl (Fmoc)], aliphatic urethane protecting groups (e.g., t-butyloxycarbonyl (Boc), isopropyloxycarbonyl, cyclohexyloxycarbonyl) and alkyl type protecting groups (e.g., benzyl, triphenylmethyl). The preferred protecting group is Boc. The side-chain protecting groups for Tyr include tetrahydropyranyl, tert.-butyl, trityl, benzyl, Cbz, 4-Br-Cbz and 2,6-dichlorobenzyl. The preferred side-chain protecting group for Tyr is 2,6-dichlorobenzyl. The side-chain protecting groups for Asp include benzyl, 2,6-dichlorobenzyl, methyl, ethyl and cyclohexyl. The preferred side-chain protecting group for Asp is cyclohexyl. The side-chain protecting groups for Thr and Ser include acetyl, benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-dichlorobenzyl and Cbz. The preferred protecting group for Thr and Ser is benzyl. The side-chain protecting groups for Arg include nitro, Tos, Cbz, adamantyloxycarbonyl and Boc. The preferred protecting group for Arg is Tos. The side-chain amino group of Lys may be protected with Cbz, 2-Cl-Cbz, Tos or Boc. The 2-Cl-Cbz group is the preferred protecting group for Lys.

The side-chain protecting groups selected must remain intact during coupling and not be removed during the deprotection of the amino-terminus protecting group or during coupling conditions. The side-chain protecting groups must also be removable upon the completion of synthesis, using reaction conditions that will not alter the finished polypeptide.

Solid phase synthesis is usually carried out from the carboxy-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl or hydroxymethyl resin, and the resulting polypeptide will have a free carboxyl group at the C-terminus. Alternatively, when a benzhydrylamine or p-methylbenzhydrylamine resin is used, an amide bond is formed and the resulting polypeptide will have a carboxamide group at the C-terminus. These resins are commercially available, and their preparation has described by Stewart *et al.*, "Solid Phase Peptide Synthesis" (2nd Edition), Pierce Chemical Co., Rockford, IL., 1984.

The C-terminal amino acid, protected at the side chain if necessary and at the alpha-amino group, is coupled to the benzhydrylamine resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide and carbonyldiimidazole. Following the attachment to the resin support, the alpha-amino protecting group is removed using trifluoroacetic acid (TFA) or HCl in dioxane at a temperature between 0 and 25°C. Dimethylsulfide is added to the TFA after the introduction of methionine (Met) to suppress possible S-alkylation. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

Various activating agents can be used for the coupling reactions including DCC, N,N'-diisopropylcarbodiimide, benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) and DCC-hydroxybenzotriazole (HOBt). Each protected amino acid is used in excess (>2.0 equivalents), and the couplings are usually carried out in N-methylpyrrolidone (NMP) or in DMF, CH₂Cl₂ or mixtures thereof. The extent of completion of the coupling reaction is monitored at each stage, e.g., by the ninhydrin reaction as described by Kaiser *et al.*, Anal. Biochem. 34:595 (1970). In cases where incomplete coupling is found, the coupling reaction is repeated. The coupling reactions can be performed automatically with commercially available instruments.

After the entire assembly of the desired polypeptide, the polypeptide-resin is cleaved with a reagent such as liquid HF for 1-2 hours at 0°C, which cleaves the polypeptide from the resin and removes all side-chain protecting groups. A scavenger such as anisole is usually used with the liquid HF to prevent cations formed during the cleavage from alkylating the amino acid residues present in the polypeptide. The polypeptide-resin may be deprotected with TFA/dithioethane prior to cleavage if desired.

Side-chain to side-chain cyclization on the solid support requires the use of an orthogonal protection scheme which enables selective cleavage of the side-chain functions of acidic amino acids (e.g., Asp) and the basic amino acids (e.g., Lys). The 9-fluorenylmethyl (Fm) protecting group for the side-chain of Asp and the 9-fluorenylmethoxycarbonyl (Fmoc) protecting group for the side-chain of Lys can be used for this purpose. In these cases, the side-chain protecting groups of the Boc-protected polypeptide-resin are selectively removed with piperidine in DMF. Cyclization is achieved on

the solid support using various activating agents including DCC, DCC/HOBt or BOP. The HF reaction is carried out on the cyclized polypeptide-resin as described above.

5 Recombinant DNA methodology can also be used to prepare the polypeptides. The known genetic code, tailored if desired for more efficient expression in a given host organism, can be used to synthesize oligonucleotides encoding the desired amino acid sequences. The phosphoramidite solid support method of Matteucci *et al.* [J. Am. Chem. Soc. 103:3185
10 (1981)], the method of Yoo *et al.* [J. Biol. Chem. 264:17078 (1989)], or other well known methods can be used for such synthesis. The resulting oligonucleotides can be inserted into an appropriate vector and expressed in a compatible host organism.

15 The polypeptides of the invention can be purified using HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution or other known methods.

20 Antibodies can be prepared against the polypeptides of the invention using standard methods. As used herein, the word "antibody" refers to both polyclonal and monoclonal antibodies.

25 The polyclonal antibodies can be produced by immunizing a host animal such as a rabbit, rat, goat, sheep, mouse, etc. with one of the polypeptides. Preferably, one or more booster injections are given after the initial injection, to increase the antibody titer. Blood is then drawn from the animal and serum is prepared and screened by standard methods such as enzyme-linked immunosorbent assay (ELISA)
30 using the polypeptide as the antigen.

Preferably, the immunogenicity of the polypeptides is increased by combination with an adjuvant and/or by conversion to a larger form prior to immunization.

Suitable adjuvants for the vaccination of animals include but are not limited to Adjuvant 65 (containing peanut oil, mannide monooleate and aluminum monostearate); Freund's complete or incomplete adjuvant; mineral gels such as aluminum hydroxide, aluminum phosphate and alum; surfactants such as hexadecylamine, octadecylamine, lysolecithin, dimethyldioctadecyl-ammonium bromide, N,N-dioctadecyl-N',N'-bis(2-hydroxymethyl) propanediamine, methoxyhexadecylglycerol and pluronic polyols; polyanions such as pyran, dextran sulfate, poly IC, polyacrylic acid and carbopol; peptides such as muramyl dipeptide, dimethylglycine and tuftsin; and oil emulsions. The polypeptides could also be administered following incorporation into liposomes or other microcarriers.

The immunogenicity of the polypeptides can also be enhanced by cross-linking or by coupling to an immunogenic carrier molecule (i.e., a macromolecule having the property of independently eliciting an immunological response in a host animal, to which the polypeptides of the invention can be covalently linked). Cross-linking or conjugation to a carrier molecule may be required because small polypeptides sometimes act as haptens (molecules which are capable of specifically binding to an antibody but incapable of eliciting antibody production, i.e., they are not immunogenic). Conjugation of such polypeptides to an immunogenic carrier molecule renders the fragments immunogenic through what is commonly known as the "carrier effect".

Suitable carrier molecules include, e.g., proteins and natural or synthetic polymeric compounds such as polypeptides, polysaccharides, lipopolysaccharides etc. A useful carrier is a glycoside called Quil A. which has been described by Morein *et al.*, Nature 308:457 (1984). Protein carrier molecules are especially preferred, including but not limited to keyhole limpet hemocyanin and mammalian serum proteins such as human or bovine gammaglobulin, human, bovine or rabbit serum albumin, or methylated or other derivatives of such proteins. Other protein carriers will be apparent to those skilled in the art. Preferably, but not necessarily, the protein carrier will be foreign to the host animal in which antibodies against the polypeptides are to be elicited.

Covalent coupling to the carrier molecule can be carried out using methods well known in the art, the exact choice of which will be dictated by the nature of the carrier molecule used. When the immunogenic carrier molecule is a protein, the polypeptides of the invention can be coupled, e.g., using water soluble carbodiimides such as dicyclohexylcarbodiimide or glutaraldehyde.

Coupling agents such as these can also be used to cross-link the polypeptides to themselves without the use of a separate carrier molecule. Such cross-linking into aggregates can also increase immunogenicity.

Serum produced from animals thus immunized can be used directly. Alternatively, the IgG fraction can be separated from the serum using standard methods such as plasmaphoresis or adsorption chromatography using IgG-specific adsorbents such as immobilized Protein A.

Monoclonal antibodies can be prepared using standard methods, e.g., as described by Kohler *et al.* [Nature 256:495 (1975); Eur. J. Immunol. 6:511 (1976)]. Essentially, an animal is immunized as described above to produce
5 antibody-secreting somatic cells. These cells are then removed from the immunized animal for fusion to myeloma cells.

Somatic cells with the potential to produce antibodies, particularly B cells, are suitable for fusion with a
10 myeloma cell line. These somatic cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals.

Specialized myeloma cell lines have been developed from lymphocytic tumors for use in hybridoma-producing fusion procedures [Kohler and Milstein, Eur. J. Immunol. 6:511 (1976); Shulman *et al.*, Nature 276:269 (1978); Volk *et al.*, J. Virol. 42:220 (1982)]. These cell lines have been developed for at least three reasons. The first is to facilitate the selection of fused hybridomas from unfused and
20 similarly indefinitely self-propagating myeloma cells. Usually, this is accomplished by using myelomas with enzyme deficiencies that render them incapable of growing in certain selective media that support the growth of hybridomas. The second reason arises from the inherent ability of lymphocytic
25 tumor cells to produce their own antibodies. The purpose of using monoclonal techniques is to obtain fused hybrid cell lines with unlimited life spans that produce the desired single antibody under the genetic control of the somatic cell component of the hybridoma. To eliminate the production of
30 tumor cell antibodies by the hybridomas, myeloma cell lines incapable of producing light or heavy immunoglobulin chains or deficient in antibody secretion mechanisms are used. A

third reason for selection of these cell lines is for their suitability and efficiency for fusion.

Many myeloma cell lines may be used for the production of fused cell hybrids, including, e.g., P3X63-Ag8, P3/NS1-Ag4-1 (NS-1), Sp2/0-Ag14 and S194/5.XXO.Bu.1. The P3X63-Ag8 and NS-1 cell lines have been described by Kohler and Milstein [Eur. J. Immunol. 6:511 (1976)]. Shulman *et al.* [Nature 276:269 (1978)] developed the Sp2/0-Ag14 myeloma line. The S194/5.XXO.Bu.1 line was reported by Trowbridge [J. Exp. Med. 148:313 (1979)].

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually involve mixing somatic cells with myeloma cells in a 10:1 proportion (although the proportion may vary from about 20:1 to about 1:1), respectively, in the presence of an agent or agents (chemical, viral or electrical) that promotes the fusion of cell membranes. Fusion methods have been described by Kohler and Milstein, *supra*, Geffer *et al.* [Somatic Cell Genet. 3:231 (1977)], and Volk *et al.* (J. Virol. 42:220 (1982)). The fusion-promoting agents used by those investigators were Sendai virus and polyethylene glycol (PEG).

Because fusion procedures produce viable hybrids at very low frequency (e.g., when spleens are used as a source of somatic cells, only one hybrid is obtained for roughly every 1×10^5 spleen cells), it is essential to have a means of selecting the fused cell hybrids from the remaining unfused cells, particularly the unfused myeloma cells. A means of detecting the desired antibody-producing hybridomas among other resulting fused cell hybrids is also necessary.

30

Generally, the selection of fused cell hybrids is accomplished by culturing the cells in media that support the growth of hybridomas but prevent the growth of the unfused myeloma cells, which normally would go on dividing indefinitely. The somatic cells used in the fusion do not maintain long-term viability in *in vitro* culture and hence do not pose a problem. In the example of the present invention, myeloma cells lacking hypoxanthine phosphoribosyl transferase (HPRT-negative) were used. Selection against these cells is made in hypoxanthine/aminopterin/thymidine (HAT) medium, a medium in which the fused cell hybrids survive due to the HPRT-positive genotype of the spleen cells. The use of myeloma cells with different genetic deficiencies (drug sensitivities, etc.) that can be selected against in media supporting the growth of genotypically competent hybrids is also possible.

Several weeks are required to selectively culture the fused cell hybrids. Early in this time period, it is necessary to identify those hybrids which produce the desired antibody, so that they may subsequently be cloned and propagated. Generally, around 10% of the hybrids obtained produce the desired antibody, although a range of from about 1 to about 30% is not uncommon. The detection of antibody-producing hybrids can be achieved by any one of several standard assay methods, including enzyme-linked immunoassay and radioimmunoassay techniques which have been described in the literature [see, e.g., Kennet *et al.* (editors), *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses*, pp. 376-384, Plenum Press, New York (1980)].

Once the desired fused cell hybrids have been selected and cloned into individual antibody-producing cell lines, each cell line may be propagated in either of two standard ways. A suspension of the hybridoma cells can be
5 injected into a histocompatible animal. The injected animal will then develop tumors that secrete the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can be tapped to provide monoclonal antibodies in high concentration.
10 Alternatively, the individual cell lines may be propagated *in vitro* in laboratory culture vessels. The culture medium containing high concentrations of a single specific monoclonal antibody can be harvested by decantation, filtration or centrifugation.

15 The anti-idiotypic antibodies of the invention are directed against antibodies specific for the gamma interferon antigenic determinants present in some of the polypeptides of the invention. Such anti-idiotypic antibodies mimic or act like the original antigenic determinants (see, e.g., U.S. Patent
20 No. 4,731,237 to Reagan *et al.*). Like gamma interferon itself, these antibodies are presumed to bind specifically and directly to gamma interferon receptors.

Such anti-idiotypic antibodies are prepared by vaccinating an animal with an antibody (polyclonal or
25 monoclonal) against a polypeptide of the invention. They may be recovered as a whole polyclonal antiserum or as an IgG or other fraction thereof, or as monoclonal antibodies produced by cloned hybridomas, as described above.

The antagonistic effects of the polypeptides and
30 antibodies of this invention can readily be demonstrated by routine experimentation using the Daudi cell radioligand-receptor assay system described below.

Recombinant human gamma interferon which can be used in this system can be made by standard recombinant methods and is also available commercially, e.g., from Genzyme Corp., Boston, MA. Such interferon can readily be labeled with
5 iodine-125 using, e.g., the lactoperoxidase method [David *et al.*, *Biochemistry* 13:1014 (1974)] or the method of Bolton *et al.* [*Biochem. J.* 133:529 (1973)].

The present invention also encompasses binding fragments of the above-mentioned polyclonal and monoclonal
10 antibodies, such as Fab, F(ab')₂, Fv fragments etc. Such fragments are obtained by conventional techniques including but not limited to papain or pepsin digestion of the intact antibodies. Of course, DNA prepared from hybridomas secreting the monoclonal antibodies of the invention can be
15 manipulated using known recombinant DNA techniques to produce chimeric antibodies or fragments thereof. The antibodies can also be "humanized" as described by Verhoeven *et al.* [*Science* 239:1534 (1988)], Reichmann *et al.* [*Nature* 332:323 (1988)] and Jones *et al.* [*Nature* 321:522 (1986)].

20 Pharmaceutical compositions can be prepared which contain effective amounts of one or more of the polypeptides or antibodies of the invention and a physiologically acceptable carrier. Such carriers are well known to those skilled in the art. The polypeptides and
25 proteins can be administered directly or in the form of a composition to a human patient afflicted, e.g., by autoimmune disease, MS or another disease mediated by gamma interferon.

Determination of the proper dosage of a polypeptide or antibody of the invention for a particular
30 situation is within the skill of the art. Generally, treatment is initiated with smaller dosages that are less than optimum. Thereafter, the dosage is increased by small increments until

the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

5 The amount and frequency of administration of the polypeptides and proteins of the invention and the pharmaceutically acceptable salts thereof will be regulated according to the judgment of the attending clinician, taking into account such factors as age, condition and size of the patient and severity of the symptom(s) being treated.

10

EXAMPLES

Unless otherwise specified, percentages given below for solids in solid mixtures, liquids in liquids, and solids in liquids are on a wt/wt, vol/vol and wt/vol basis, respectively.

15

Protein Analysis

Protein determinations were carried out by the method of Lowry *et al.* [J. Biol. Chem. 193:265 (1951)] using bovine serum albumin as a standard.

Sources of Polypeptides and Proteins

20

Polypeptides having amino acid sequences corresponding to various regions of mature human gamma interferon A were synthesized using the solid-phase method of Merrifield [J. Am. Chem. Soc. 85:2149 (1963)]. The t-butyloxycarbonyl amino protecting group, symmetrical anhydrides, and an Applied Biosystems Model 430A
25 solid-phase peptide synthesizer were employed. Following removal of protecting groups, the polypeptides were cleaved from the resin with hydrogen fluoride. Crude polypeptides recovered after cleavage from the resin were analyzed by

reverse-phase HPLC in a Rainin Dynamax® C-8 column (12 μ particle size, 300 Angstrom pore size, 4.6 X 250 mm).

In this way, the following polypeptides were prepared:

- 5 Lys-Lys-Tyr-Phe-Asn-Ala-Gly-Gly-Gly-Gly-Arg-Lys-Arg-Ser-Gln-Met-Leu (15-21-Gly-Gly-Gly-132-138; SEQ ID NO:2)
- Arg-Lys-Arg-Ser-Gln-Met (132-137; SEQ ID NO:3)
- 10 Cys-Tyr-Cys-Gln-Asp-Pro-Tyr-Val-Lys (1-9; SEQ ID NO:4)
- Asp-Tyr-Val-Lys-Glu-Ala-Glu-Asn-Leu-Lys-Lys-Tyr-Phe-Asn (Asp 7-19; SEQ ID NO:5)
- Leu-Ile-Gln-Val-Met-Ala-Glu-Leu-Ser-Pro-Ala-Ala-Lys-Thr-Gly (116-130; SEQ ID NO:6)
- 15 Leu-Ser-Pro-Ala-Ala-Lys-Thr-Gly-Lys-Arg-Lys-Arg-Ser-Gln-Met (123-137; SEQ ID NO:7)
- Gly-Ile-Leu-Lys-Asn-Trp-Lys-Glu-Glu-Ser-Asp-Arg-Lys-Ile-Cys (34-47 Cys; SEQ ID NO:8)
- 20 Val-Gln-Arg-Lys-Ala-Ile-His-Glu-Leu-Ile-Gln-Val-Met-Ala-Glu (108-122; SEQ ID NO:9)
- Lys-Lys-Tyr-Phe-Asn-Ala-Gly-His-Ser-Asp-Val-Ala-Asp-Asn-Gly-Lys-Ala-Ile-His-Glu-Leu-Ile-Gln-Gly-Lys-Arg-Lys-Arg-Ser-Gln-Met-Leu (15-29, 111-118, 130-138; SEQ ID NO:10)

- 25 The sequence of each polypeptide shown above is followed in parentheses by the corresponding human gamma interferon A residues (see SEQ ID NO:1) upon which the sequence was based, and the corresponding SEQ ID NO. assigned to the polypeptide in the Sequence Listing. Note that
- 30 in some cases the sequences contain additional amino acid residues as indicated.

Recombinant human gamma interferons A and D were prepared and purified from transformed *E. coli*, essentially as described in U.S. patent No. 4,751,078.

Preparation of Antisera

5 Anti-polypeptide Antibodies

Five-hundred μ l volumes of aqueous pH 7.1 solutions containing 0.5 to 1.0 mg of the various polypeptides were emulsified with equal volumes of Freund's complete adjuvant and injected intradermally (0.1 ml per injected site) into New Zealand White rabbits (Hazelton Labs). Booster injections containing about 0.25 to 0.5 mg of polypeptide in Freund's incomplete adjuvant were administered at approximately 4-week intervals as required, as judged by ELISA analyses of serum samples using the polypeptides and gamma interferon as antigens.

Antibodies against the discontinuous polypeptide were purified commercially by adsorption to a Protein A-Sepharose® column equilibrated with 1.5 M glycine buffer, pH 8.9. Analysis by SDS polyacrylamide gel electrophoresis [Laemmli, Nature 227:680 (1970)] showed that the antibody preparation thereby obtained was 95% pure IgG.

Anti-idiotypic Antibodies

The purified rabbit IgG antibodies against the discontinuous polypeptide were used to immunize Balb/c mice (Charles River Labs). The mice were primed intraperitoneally with 0.5 ml of pristane (Sigma Chemical Co., St. Louis, MO) 6 days prior to immunization by the same route with 87 μ g of the IgG protein in 125 μ l of phosphate buffered saline and 125 μ l of Freund's complete adjuvant. Boosts of approximately 50 μ g of the protein in 50% Freund's

incomplete adjuvant were given at approximately 2-3 week intervals as needed, based upon ELISA analyses.

ELISA analysis of the rabbit antisera was carried out at room temperature. A 96-well microtiter plate (Nunc) was coated with 0.25 μ g of antigen in 100 μ l per well for 1 hour at room temperature. The plate was washed 5 times with tris-buffered saline (TBS), pH 7.5, containing 0.05% Tween 20 (polyoxethylenesorbitan monolaurate). The plate was blocked with 1% bovine serum albumin for 1 hour, washed 5-times with TBS and again washed 5 times with TBS.

The wells of the washed plate were coated with antisera to be tested for 1 hour, washed 5 times with TBS and coated with 2.5 mg of horseradish peroxidase-conjugated goat anti-rabbit IgG. Following incubation for 1 hour, the plate was washed 5 times with TBS and developed by adding either 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) or 3,3',5,5'-Tetramethylbenzidine (TMB) and hydrogen peroxide to each well. Color development was stopped after 10 minutes by adding a solution containing sulfuric acid (for TMB) or sodium dodecylsulfate (for ABTS), and the samples were read at 405 and 450 nm for ABTS and TMB, respectively, using a Molecular Devices ELISA reader.

ELISA analysis of mouse serum was performed in essentially the same way except that peroxidase-labeled goat anti-mouse IgG was used as the detection reagent.

Labeling of Human Recombinant Gamma Interferon

Recombinant human gamma interferon D was purified to homogeneity from an *E. coli* lysate essentially as described in U.S. Patent No. 4,751,078, although similar interferon is available commercially from sources such as Genzyme Corp., Boston, MA.

The interferon was labeled with iodine-125, essentially as described by Bolton *et al.* [Biochem. J. 133:529 (1973)], using ^{125}I , Bolton-Hunter reagent from New England Nuclear, Boston, MA. Briefly 2 mCi (2200 Ci/mmol) of Bolton-Hunter reagent (New England Nuclear, Boston, MA) in anhydrous benzene was dried by a gentle stream of nitrogen. Five micrograms of gamma interferon dissolved in 50 μl of 50 mM sodium phosphate buffer, pH 8.0, were added to the reaction vessel. The reaction was allowed to proceed for 2 hours at room temperature, after which unreacted Bolton-Hunter reagent was quenched with 50 μl of 1 M glycine in 50 mM sodium phosphate, pH 8.0.

The radioiodinated protein was separated from unreacted labeling reagent by gel filtration in a 10 ml PD-10 Sephadex G-25[®] column (Pharmacia LKB Biotechnology, Piscataway, NJ) equilibrated with 0.05 M sodium phosphate buffer, pH 7.2, containing 0.5% gelatin. The same buffer was used to develop the column. One-ml fractions were collected, and aliquots were counted in a gamma counter. Labeled protein eluted in the column void volume (3-4 ml), while unreacted reagent eluted later (6-8 ml).

The labeled interferon typically had a specific radioactivity of 50 to 200 $\mu\text{Ci}/\mu\text{g}$. Analysis by SDS polyacrylamide gel electrophoresis followed by autoradiography showed that the labeled protein migrated as a single band with a mobility essentially identical to that of unlabeled gamma interferon.

Inhibition of the Binding of ^{125}I -labeled Human Gamma Interferon D to Daudi Cells

Daudi cell stock cultures were grown in RPMI 1640 medium containing 100 units/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 2 mM glutamine and 20% heat-inactivated fetal

bovine serum. The cells were grown in T-75 flasks (50 ml/flask), seeded at 4×10^5 cells/ml and split at 2-day intervals (or seeded at 2.5×10^5 cells/ml for 3-day growth over weekends). The cultures were maintained during growth at 4°C.

The cells were harvested for assay by centrifugation at $300 \times g$ for 3 minutes at 4°C. The spent culture medium was discarded and the cell pellets were suspended in ice-cold binding buffer (RPMI 1640, 10% heat-inactivated fetal bovine serum, 15 mM HEPES, 0.02% sodium azide). Cells were counted by hemocytometer, viability was established by percent trypan blue exclusion [Animal Cell Culture: A Practical Approach, 1986, R.I. Freshney (Ed.), IRL Press, LTD, pp. 76-77], and the cells were suspended at 2×10^6 cells/ml in the cold binding buffer.

Binding assays were performed on polypeptides and rabbit antisera in 1-ml volumes using Sarstedt 1.5 ml screw cap conical tubes. All components were diluted in the cold binding buffer and added in the following order:

- 20 450 μ l of unknown sample (or control binding buffer only)
- 50 μ l of 125 I-labeled gamma interferon D (10^5 cpm)
- 500 μ l of cell suspension (10^6 viable cells)

In addition, 10 μ l of an unlabeled gamma interferon A solution containing 1 μ g of protein were added to some control tubes to determine the level of nonspecific binding by the labeled interferon. This amount of radioactivity was subtracted from all radioactivity measurements.

After all of the components had been added to each tube, the tubes were capped, inverted briefly to wet the entire inner surfaces and incubated for 2 hours in a cold room on an Adams Nutator® rocking platform. Following this incubation, the supernatant fluid was aspirated from each tube, taking care not to remove any cells.

One-hundred μ l of the ice-cold binding buffer was added to each tube, and the pellets were suspended using a 200 μ l Gilson Pipetman® and carefully layered atop 400 μ l of ice-cold 5% sucrose in PBS in Sarstedt 600 μ l conical tubes held in aluminum centrifugation racks. The samples were centrifuged at 1,000 X g for 5 minutes at 4°C.

The supernatant fluids were carefully aspirated to avoid loss of cells, and the tubes were transferred to 12 x 75 mm tubes suitable for gamma counter use.

Binding Inhibition By Polypeptides

The polypeptides having amino acid sequences defined by SEQ ID NO:2 and SEQ ID NO:3 were tested in the receptor binding assay as described above, with the results shown in Fig. 1.

There, it can be seen that the polypeptide defined by SEQ ID NO:2 (panel A) and the other polypeptide (panel B) both inhibited the binding of 125 I-labeled human gamma interferon D to receptors on Daudi cells. The former polypeptide was the more potent competitive inhibitor, showing binding inhibitions of about 15 and 35% at concentrations of 0.145 and 1.45 μ M, respectively.

Binding Inhibition by Antibodies

Rabbit polyclonal antisera prepared against all of the polypeptides mentioned above were tested for ability to bind to the polypeptides and to human gamma interferon A and to inhibit the specific binding of human gamma interferon D to Daudi cells, with the results shown in Table I.

Table ICharacterization of Antisera

10

Polypeptide SEQ ID NO.	% ¹²⁵ I-Interferon Binding Inhibition ^a	Specific Binding ^b to Polypeptide γ-Interferon A
2	86	+
3	-	-
4	11	+
5	76	+
6	14	+
7	65	+
8	16	+/-
9	33	+

^a Percent binding inhibitions are expressed as percentages of the specific binding shown by ¹²⁵I-labeled human gamma interferon D in the absence of any inhibitor.

15

^b Positive binding responses (+) were defined as ELISA results in which responses were more than 0.15 optical density (O.D.) units above background at . The +/- notation signifies responses that were between 0.0 and 0.15 O.D. units above background.

20

The data of Table I show that antibodies against polypeptides having amino acid sequences corresponding to the sequences of residues 7-19 (with an added N-terminal aspartate residue), 123-137 and 5-21/132-138 (with 3 bridging glycine residues) of mature human gamma interferon A (polypeptide SEQ ID NOs. 5, 7 and 2, respectively) were all antagonists of the specific binding of the labeled interferon to Daudi cell receptors. All three of these antisera contained antibodies that, as shown by ELISA, specifically bound to both the polypeptides against which they were produced and to gamma interferon A. No antibodies were produced against the 132-137 polypeptide (polypeptide SEQ ID NO:3), possibly because it was too small to be immunogenic unconjugated to a carrier molecule.

Rabbit preimmune serum was completely unreactive in all of the assays. As shown in Fig. 2, antiserum against the polypeptide defined by SEQ ID NO:2 almost completely abolished the specific binding of labeled gamma interferon D to Daudi cells at concentrations above about 25 μ l of serum per ml of incubation mixture. About 50% binding inhibition was observed at a concentration of this antiserum of only 1 μ l/ml.

The anti-idiotypic antiserum prepared using the IgG antibody fraction against the polypeptide defined by SEQ ID NO:2 also demonstrated significant receptor binding inhibition, as shown in Table II.

Table IIInhibition of ^{125}I -labeled Human Gamma Interferon
Binding To Daudi Cells by Anti-idiotypic Antiserum

5

<u>Bleed^a</u>	<u>% Specific Binding Inhibition^b</u>
first	16
first	31
first	38
second	44

10 ^a The data shown are the results obtained using antiserum taken from mice immunized as described above 16 weeks after the first injection (first bleed) and 17 weeks thereafter (second bleed). The second bleeds of the three mice were pooled to produce the result shown.

^b Results were normalized using control preimmune serum.

15 The data shown in Table II are the result of incubating 50-100 μl of mouse serum with 10^5 cpm of the ^{125}I -labeled gamma interferon and 1.5×10^6 viable Daudi cells, essentially as described above. The data show that the inhibitory ability of the anti-idiotypic antiserum increased as

20 the course of immunization progressed (i.e., the second bleed was stronger than the first).

Inhibition of the Biological
Effects of Gamma Interferon

25 To demonstrate that the polypeptides of the invention also produce biological effects, the action of a representative polypeptide was observed in an interferon-responsive biological system.

Reagents and Cells

Recombinant human gamma interferon D having a specific activity of about 5×10^6 units/mg was prepared from transformed *E. coli* using standard methods.

- 5 COLO-205 cells (ATCC CLL 222) derived from a human adenocarcinoma were used to measure the induction by the interferon of class II major histocompatibility antigens (HLA-DR). The presence of the antigens on the cells was detected by Enzyme-Linked Immunosorbent Assay (ELISA)
10 using a mouse monoclonal anti-HLA-DR antibody (Becton-Dickinson Catalog No. 7360) in conjunction with a peroxidase-labeled goat anti-mouse IgG. Color produced using 2,2'-Azino-bis(3-Ethylbenzthiazoline-6-Sulfonic Acid) (ABTS; Kirkegaard & Perry Labs., Inc., Gaithersburg, MD) was
15 measured spectrophotometrically at 405 nm.

Inhibition of HLA-DR Induction

- Bio-ELISA assay for HLA-DR induction by gamma interferon was carried out essentially as described by Gibson *et al.* [J. Immunol. Meth. 125:103 (1989)]. Briefly, COLO-205
20 cells were grown to confluence in T-75 flasks in RPMI 1640 medium containing 10% fetal calf serum (culture medium). The cells were trypsinized and seeded in 96-well tissue culture plates at a density of at least 10^5 cells per well in 0.1 ml of culture medium. The cells were allowed to attach to
25 the wells by overnight incubation at 37°C in a 5% CO₂ incubator.

- Control culture medium and various dilutions in culture medium of the polypeptide defined by SEQ ID NO:10 in the presence of a fixed 150 pM concentration of the interferon
30 were added in a 0.1 ml volume to the wells and incubated for one hour at 37°C.

Following this incubation, the medium was removed from each well and the wells were washed three times with culture medium. Aliquots (0.1 ml) of culture medium were added to the wells, and the plates were
5 incubated for 48 hours at 37°C to allow for induction of HLA-DR antigen expression by interferon bound to the cells.

The wells were washed with 0.2 ml of phosphate buffered saline (PBS; 0.02 M sodium phosphate, 0.15 M NaCl, pH 7.4) and then fixed for two minutes with ice-cold
10 anhydrous ethyl alcohol. The alcohol was removed, and the wells were washed once with 0.2 ml of PBS. Fifty microliters of a 1:50 dilution of the mouse monoclonal anti-HLA-DR antibody in PBS containing 0.5% bovine serum albumin were then added to each well, and the plates were incubated for one
15 hour at room temperature.

Excess reagent was removed by washing the wells three times with 0.2 ml of PBS, after which 0.1 ml of a 1:5,000 dilution of peroxidase-labeled goat anti-mouse IgG was added to each well. The plates were incubated for one
20 hour at room temperature. After washing each well three times with PBS as before, color was developed by the addition of ABTS for 5-10 minutes at room temperature. Absorbance was measured at 405 nm using an ELISA plate reader.

Results of an assay to measure inhibition of
25 gamma interferon-induced HLA-DR expression in COLO-205 cells by the polypeptide defined by SEQ ID NO:10 are shown in Fig. 3. There, it can be seen that increasing concentrations of the polypeptide of from 7 to 100 µM produced progressively greater inhibition of antigen induction by the constant 150 pM
30 concentration of interferon.

Many modifications and variations of this invention may be made without departing from its spirit and scope, as will become apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims.

10

15

20

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Seelig, Gail F.

(ii) TITLE OF INVENTION: Antagonists of Human Gamma Interferon

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Schering-Plough Corporation

(B) STREET: One Giralda Farms

(C) CITY: Madison

(D) STATE: New Jersey

(E) COUNTRY: USA

(F) ZIP: 07940

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: Apple Macintosh

(C) OPERATING SYSTEM: Macintosh 6.0.5

(D) SOFTWARE: Microsoft Word 4.00B

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US07/589106

(B) FILING DATE: 27-SEP-1990

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Dulak, Norman C.

(B) REGISTRATION NUMBER: 31,608

(C) REFERENCE/DOCKET NUMBER: JB0151K

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-822-7375

(B) TELEFAX: 201-822-7039

(C) TELEX: 219165

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

36

(A) LENGTH: 146 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Cys	Tyr	Cys	Gln	Asp	Pro	Tyr	Val	Lys	Glu	Ala	Glu	Asn	Leu	Lys	Lys
1				5					10					15	
Tyr	Phe	Asn	Ala	Gly	His	Ser	Asp	Val	Ala	Asp	Asn	Gly	Thr	Leu	Phe
			20					25					30		
Leu	Gly	Ile	Leu	Lys	Asn	Trp	Lys	Glu	Glu	Ser	Asp	Arg	Lys	Ile	Met
			35				40					45			
Gln	Ser	Gln	Ile	Val	Ser	Phe	Tyr	Phe	Lys	Leu	Phe	Lys	Asn	Phe	Lys
	50					55					60				
Asp	Asp	Gln	Ser	Ile	Gln	Lys	Ser	Val	Glu	Thr	Ile	Lys	Glu	Asp	Met
65					70					75				80	
Asn	Val	Lys	Phe	Phe	Asn	Ser	Asn	Lys	Lys	Lys	Arg	Asp	Asp	Phe	Glu
				85					90					95	
Lys	Leu	Thr	Asn	Tyr	Ser	Val	Thr	Asp	Leu	Asn	Val	Gln	Arg	Lys	Ala
			100					105					110		
Ile	His	Glu	Leu	Ile	Gln	Val	Met	Ala	Glu	Leu	Ser	Pro	Ala	Ala	Lys
		115					120					125			
Thr	Gly	Lys	Arg	Lys	Arg	Ser	Gln	Met	Leu	Phe	Arg	Gly	Arg	Arg	Ala
	130					135						140			
Ser	Gln														
145															

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Lys	Lys	Tyr	Phe	Asn	Ala	Gly	Gly	Gly	Gly	Arg	Lys	Arg	Ser	Gln	Met
1				5					10					15	
Leu															

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Arg	Lys	Arg	Ser	Gln	Met
1				5	

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Cys Tyr Cys Gln Asp Pro Tyr Val Lys

1

5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Asp Tyr Val Lys Glu Ala Glu Asn Leu Lys Lys Tyr Phe Asn

1

5

10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Leu	Ile	Gln	Val	Met	Ala	Glu	Leu	Ser	Pro	Ala	Ala	Lys	Thr	Gly
1				5				10					15	

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Leu	Ser	Pro	Ala	Ala	Lys	Thr	Gly	Lys	Arg	Lys	Arg	Ser	Gln	Met
1				5				10					15	

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Gly	Ile	Leu	Lys	Asn	Trp	Lys	Glu	Glu	Ser	Asp	Arg	Lys	Ile	Cys
1				5					10					15

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Val	Gln	Arg	Lys	Ala	Ile	His	Glu	Leu	Ile	Gln	Val	Met	Ala	Glu
1				5					10					15

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Lys	Lys	Tyr	Phe	Asn	Ala	Gly	His	Ser	Asp	Val	Ala	Asp	Asn	Gly	Lys
1				5				10					15		
Ala	Ile	His	Glu	Leu	Ile	Gln	Gly	Lys	Arg	Lys	Arg	Ser	Gln	Met	Leu
			20					25					30		

WHAT IS CLAIMED IS:

1. A polypeptide containing up to 50 amino acid residues and comprising one or more amino acid subsequences selected from the group consisting of the subsequences
5 defined by amino acid residues 15-21 and 132-137 of the sequence defined by SEQ ID NO:1.
2. The polypeptide of claim 1 which has an amino acid sequence defined by SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:10.
3. An antibody or antibody binding fragment against a
10 polypeptide of either claim 1 or 2.
4. An anti-idiotypic antibody or antibody binding fragment against an antibody of claim 3.
5. A method for inhibiting the binding of human gamma interferon to cellular receptors comprising contacting cells
15 bearing receptors for human gamma interferon with an effective amount of a polypeptide of either claim 1 or 2, an antibody or binding fragment of claim 3, or an anti-idiotypic antibody or binding fragment of claim 4.
6. A pharmaceutical composition for treating diseases
20 mediated by gamma interferon comprising a physiologically acceptable carrier and an effective amount of a polypeptide of either claim 1 or 2, an antibody or binding fragment of claim 3, or an anti-idiotypic antibody or binding fragment of claim 4.
7. Use of a polypeptide of either claim 1 or 2, an
25 antibody or binding fragment of claim 3, or an anti-idiotypic antibody or binding fragment of claim 4 for the manufacture of a medicament for treating diseases mediated by gamma interferon.

8. A method for making a pharmaceutical composition for treating diseases mediated by gamma interferon comprising admixing a physiologically acceptable carrier and an effective amount of a polypeptide of either claim 1 or 2, an antibody or binding fragment of claim 3, or an anti-idiotypic antibody or binding fragment of claim 4.

9. Use of a polypeptide of either claim 1 or 2, an antibody or binding fragment of claim 3, or an anti-idiotypic antibody or binding fragment of claim 4 for treating diseases mediated by gamma interferon.

10. A method for treating diseases mediated by gamma interferon comprising administering to a patient afflicted with such a disease an effective amount of a polypeptide of either claim 1 or 2, an antibody or binding fragment of claim 3, or an anti-idiotypic antibody or binding fragment of claim 4.

1 / 2

FIG. 1A

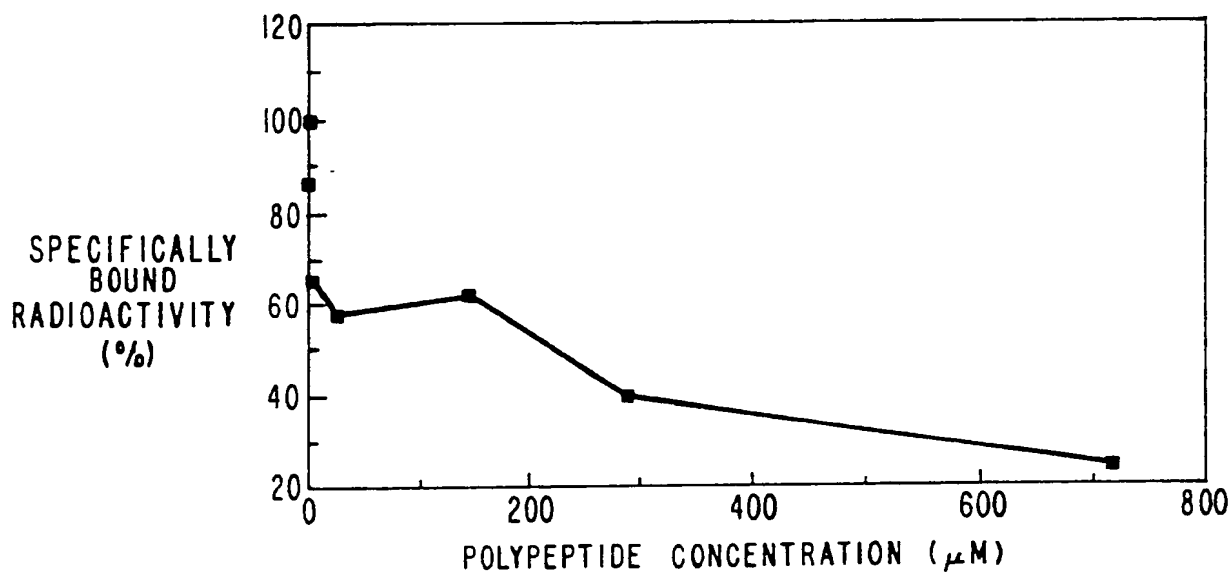


FIG. 1B

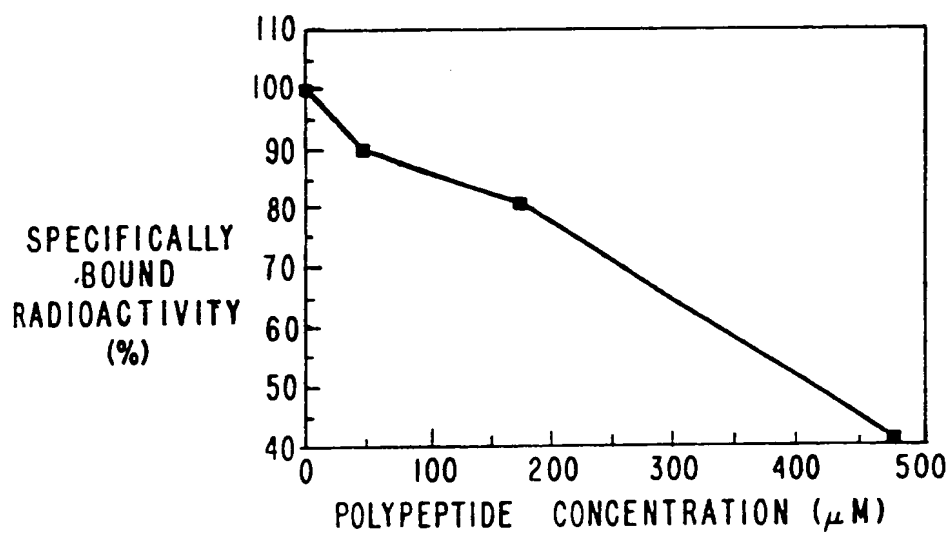


FIG. 2

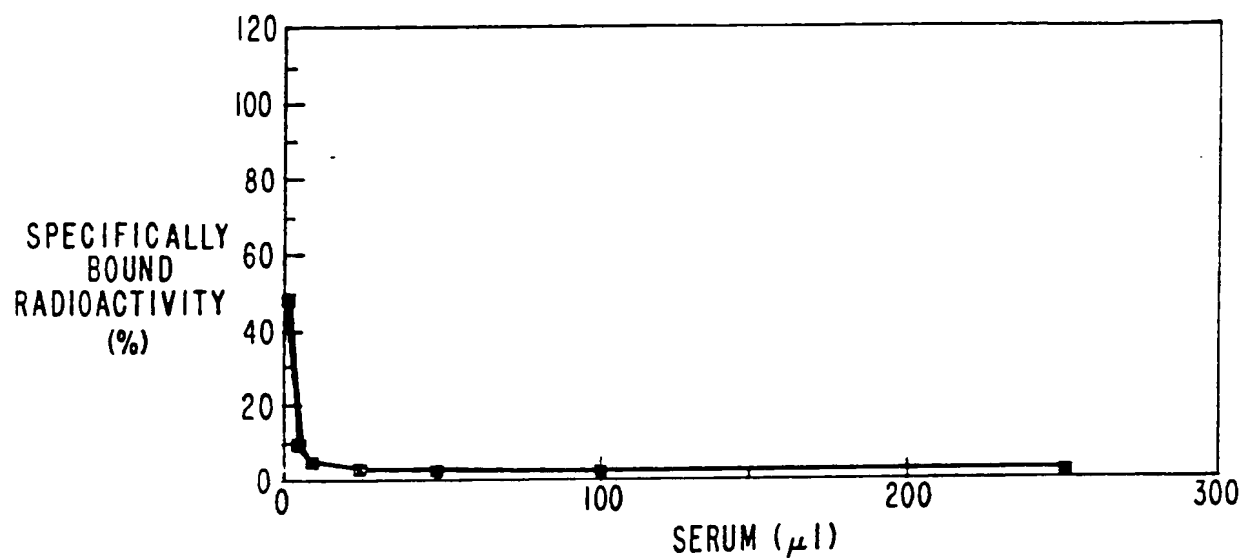
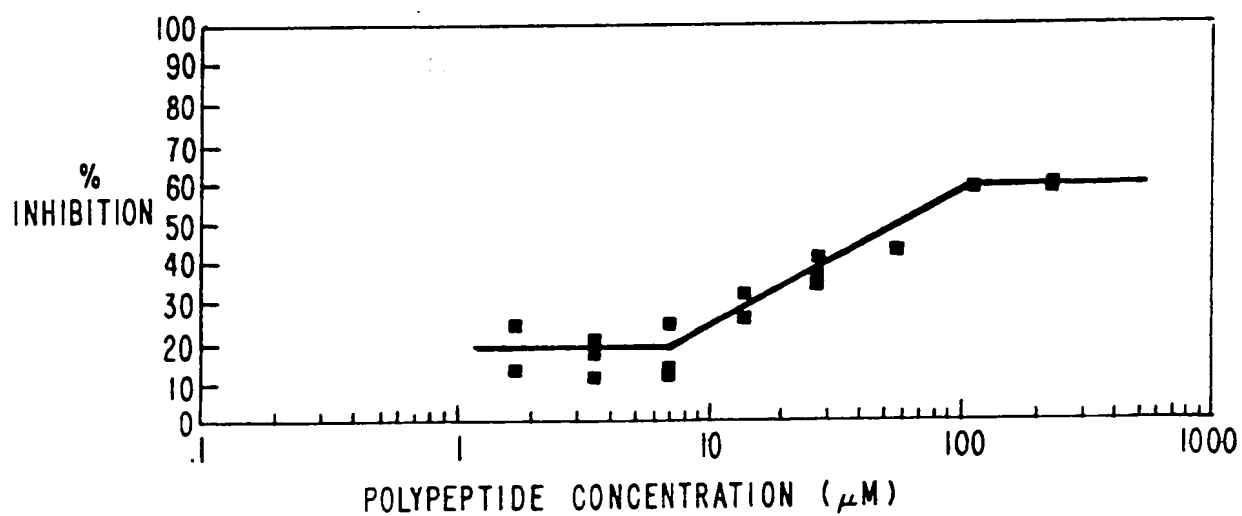


FIG. 3



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 91/06771

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 07 K 13/00, 7/10, 7/08, 15/28, A 61 K 39/395																	
II. FIELDS SEARCHED <div style="text-align: right; font-size: small;">Minimum Documentation Searched⁷</div> <table style="width: 100%; border: none;"> <tr> <td style="width: 25%; border: none;">Classification System</td> <td style="border: none;">Classification Symbols</td> </tr> <tr> <td style="border: 1px solid black; height: 40px; vertical-align: middle;">IPC5</td> <td style="border: 1px solid black; vertical-align: middle;">C 07 K; A 61 K; C 12 P</td> </tr> </table> <div style="text-align: center; font-size: x-small; margin-top: 5px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched⁸</div>			Classification System	Classification Symbols	IPC5	C 07 K; A 61 K; C 12 P											
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III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table style="width: 100%; border: none;"> <tr> <th style="width: 10%; border: none;">Category *</th> <th style="width: 70%; border: none;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 20%; border: none;">Relevant to Claim No.¹³</th> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">P,X</td> <td style="border: 1px solid black; vertical-align: top;">WO, A1, 9102005 (TURANO ADOLFO) 21 February 1991, see especially page 32 <div style="text-align: center;">--</div></td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-9</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">X</td> <td style="border: 1px solid black; vertical-align: top;">EP, A2, 0122628 (AMGEN) 24 October 1984, see especially pages 10-11 <div style="text-align: center;">--</div></td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-9</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">X</td> <td style="border: 1px solid black; vertical-align: top;">EP, A1, 0103898 (TAKEDA CHEMICAL INDUSTRIES, LTD.) 28 March 1984, see claim 1 <div style="text-align: center;">--</div></td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-9</td> </tr> <tr> <td style="border: 1px solid black; text-align: center; vertical-align: top;">X</td> <td style="border: 1px solid black; vertical-align: top;">Chemical Abstracts, volume 104, no. 25, 23 June 1986, (Columbus, Ohio, US), see page 172, abstract 220125a, & JP, A, 6041697 (Physiologically active polypeptide manufacture by recombinant DNA technology) 1985 <div style="text-align: center;">--</div></td> <td style="border: 1px solid black; text-align: center; vertical-align: top;">1-9</td> </tr> </table>			Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	P,X	WO, A1, 9102005 (TURANO ADOLFO) 21 February 1991, see especially page 32 <div style="text-align: center;">--</div>	1-9	X	EP, A2, 0122628 (AMGEN) 24 October 1984, see especially pages 10-11 <div style="text-align: center;">--</div>	1-9	X	EP, A1, 0103898 (TAKEDA CHEMICAL INDUSTRIES, LTD.) 28 March 1984, see claim 1 <div style="text-align: center;">--</div>	1-9	X	Chemical Abstracts, volume 104, no. 25, 23 June 1986, (Columbus, Ohio, US), see page 172, abstract 220125a, & JP, A, 6041697 (Physiologically active polypeptide manufacture by recombinant DNA technology) 1985 <div style="text-align: center;">--</div>	1-9
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents:¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 50%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>																	
IV. CERTIFICATION <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none;"> Date of the Actual Completion of the International Search 27th January 1992 </td> <td style="width: 50%; border: none;"> Date of Mailing of this International Search Report 11. 02. 92 </td> </tr> <tr> <td style="border: none;"> International Searching Authority EUROPEAN PATENT OFFICE </td> <td style="border: none;"> Signature of Authorized Officer T. MORTENSEN </td> </tr> </table>			Date of the Actual Completion of the International Search 27th January 1992	Date of Mailing of this International Search Report 11. 02. 92	International Searching Authority EUROPEAN PATENT OFFICE	Signature of Authorized Officer T. MORTENSEN											
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages .	Relevant to Claim No
X	Chemical Abstracts, volume 107, no. 1, 6 July 1987, (Columbus, Ohio, US), Ichimori Yuzo et al: "Monoclonal antibodies to human interferon- γ . II: Antibodies with neutralizing activity", see page 513, abstract 5451k, & Hybridoma 1987, 6(2), 173- 181 ----- -----	1-9

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1. ☒ Claim numbers 10 because they relate to subject matter not required to be searched by this Authority, namely:
See PCT rule 39.1(iv): methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.
2. ☐ Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the international searching authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 91/06771

SA 52508

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 31/10/91. The European Patent office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9102005	21/02/91	AU-D- 6176690	11/03/91
EP-A2- 0122628	24/10/84	CA-A- 1240939	23/08/88
		EP-A- 0462623	27/12/91
		US-A- 4599306	08/07/86
		WO-A- 84/04172	25/10/84
EP-A1- 0103898	28/03/84	JP-A- 57197659	03/12/82
		WO-A- 84/01149	29/03/84
		WO-A- 84/02130	07/06/84
		JP-C- 1152341	30/06/83
		JP-A- 51012467	31/01/76
		JP-B- 57046883	06/10/82
		WO-A- 84/04745	06/12/84

For more details about this annex : see Official Journal of the European patent Office, No. 12/82

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